

24. Polymerase Chain Reaction (PCR)

*The in vitro amplification of DNA by repeated cycles of strand separation and polymerization, by DNA polymerase activity, is called **polymerase chain reaction (PCR)**. In each polymerization, the number of DNA fragments gets doubled. It makes millions or billions of copies from a single DNA within a short time, i.e. less than 5 hours. The PCR was invented by **Kary Mullis** in 1985.*

*PCR is a technique of **gene amplification**. Making more copies of DNA from a single copy of DNA is called **gene amplification**.*



Fig.24.1: A PCR machine for the amplification of DNA.

Instrumentation

PCR is carried out in a single *tube* kept in a *thermal cylinder* which is programmable to set alternate heating and cooling. The PCR requires the following:

The DNA to be amplified,

Two types of oligonucleotide primers,

Deoxyribonucleotides,

Taq polymerase enzymes.

All these are added into the tube. Over this mixture, a thin film of *mineral oil* is poured to prevent evaporation of the reaction mixture during thermal cycles. The tube is then kept inside the *thermal cylinder*. This is the *experimental setup* for PCR.

Principle and Method

In PCR, the DNA to be amplified acts as *template* strands for DNA polymerization. The reagents include *Tris-HCl buffer*, *magnesium chloride* and *potassium chloride*.

The tris-HCl buffer maintains the pH between 6.8 and 7.8 during the thermal cycles.

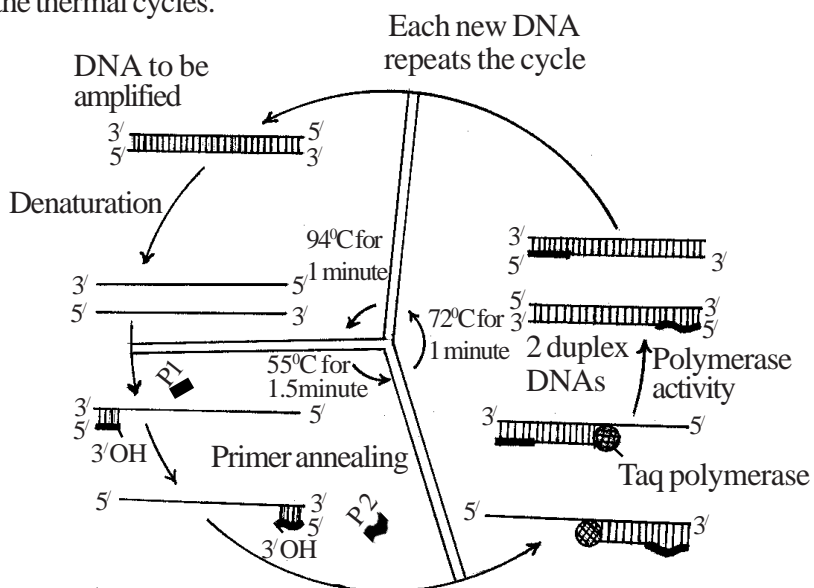


Fig.24.2: A single cycle of polymerase chain reaction.

P1=Primer1; P2=Primer 2

Magnesium and *potassium* act as *co-factors* for the enzyme reaction.

The deoxyribonucleotides include *dATP*, *dGTP*, *dCTP* and *dTTP*. Each *primer* attaches with one of the strands of the DNA and provides a *3'-OH group* for polymerization.

The thermal cylinder is programmed in such a way as to provide cycles of *94°C for 1 minute*, *55°C for 1.5 minutes* and *72°C for 1 minute* to the reaction tube.

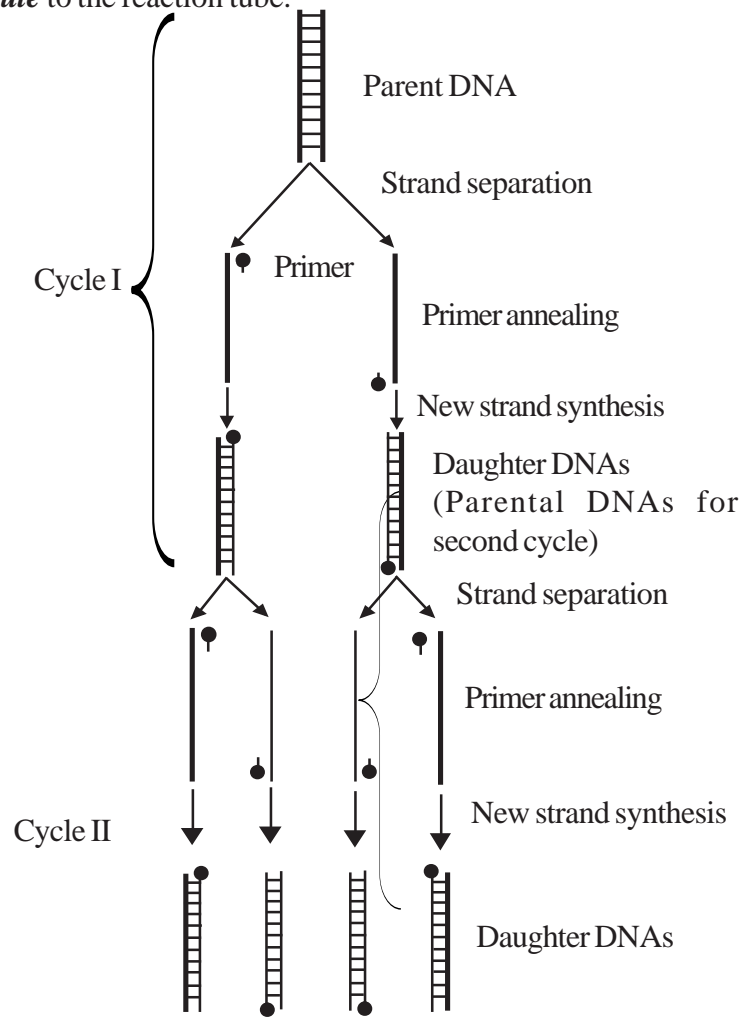


Fig.24.3: Steps in PCR (only two cycles of PCR are shown).

In the *first step*, the reaction mixture is heated to 94°C for 1 minute. This temperature *denatures* the double stranded DNA into two individual strands.

In the *second step*, the reaction mixture is cooled slowly to 55°C for 1.5 minutes. During this time, one *primer* binds with 3' end of one DNA strand and the other primer binds with the 3' end of its complementary strand. This process is called *primer annealing*.

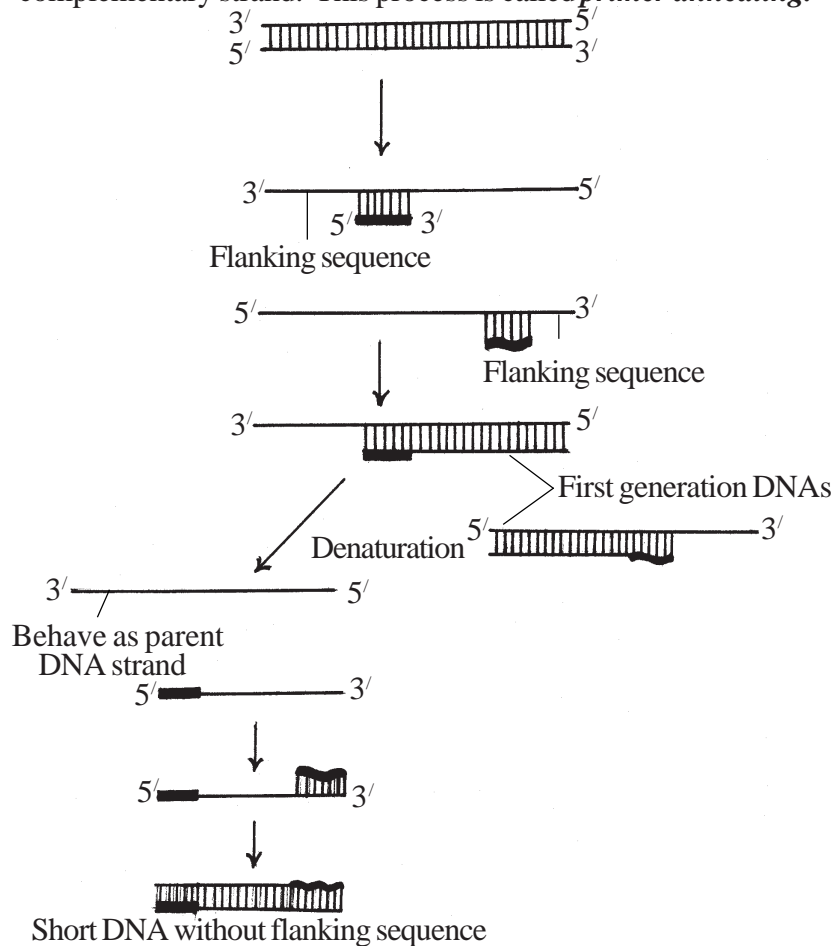


Fig.24.4: Shortening of DNA during PCR..

In the *third step*, 72°C temperature is provided to the reaction mixture for 1 minute. As this temperature is suitable for *Taq DNA polymerase*, it adds complementary nucleotides one by one to the 3'-OH group of the primer. As a result, the second DNA strand grows to the full size. This process is called *polymerase activity*.

At the end of the first cycle, two identical double stranded DNAs are formed from a DNA in the reaction mixture. Each cycle completes within 5 minutes.

Each of the two duplex DNAs formed in the first cycle enters into the second cycle. The cycle is repeated several times.

The basic reactions of PCR are the same in all the cycles. In each cycle, the number of duplex DNAs gets doubled, i.e. $2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow 32 \rightarrow 64 \rightarrow 128$ and so on. By the 30th cycle, the DNA forms about million copies.

If the primer fits exactly at the 3' end of DNA strand without leaving flanking sequence, all copies of the DNA are similar in length. If it binds on the DNA just behind the 3' end and leaves a flanking sequence, the flanking sequence has not been polymerized. So the copy DNAs are slightly shorter than the original DNA.

PCR gives good result if the DNA is less than 300 bp in size. If there is a need to amplify still larger DNA, its small units are amplified separately and then joined together.

Applications of PCR

PCR is very specific and accurate so that the final product (DNA) is pure and identical. It can be adopted to amplify DNA, even if it is available in a small amount. PCR has the following applications :

1. PCR is used to propagate DNA for gene manipulation and for constructing DNA libraries.
2. It is used to amplify DNA fragments isolated from organisms. The amplified DNAs are used for the clinical diagnosis of genetic diseases and pathogenic diseases.
3. It is used to propagate DNAs to detect the sex of the baby even at **8-16 celled stage**. This technique is used in *in vitro* fertilized eggs.
4. PCR is employed for the amplification of DNA to detect the criminals in forensic science.
5. Single sided PCR is used to make gene probes to detect genetic diseases, pathogenic diseases and to detect the presence of a particular gene in the sample.
6. Inverse PCR is used in chromosome walking to detect the correct order of arrangement of various genes in a chromosome.
7. Random PCR is used to amplify a particular DNA in a pool of DNAs.
8. RT-PCR makes cDNA copies selectively from a single species mRNA found in a pool of mRNAs.